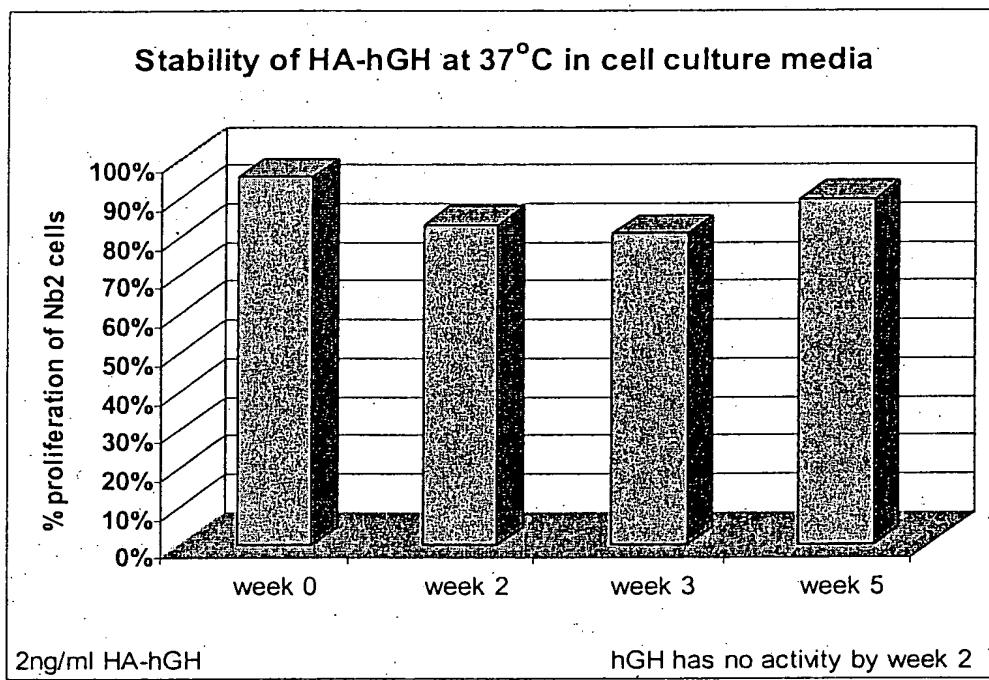
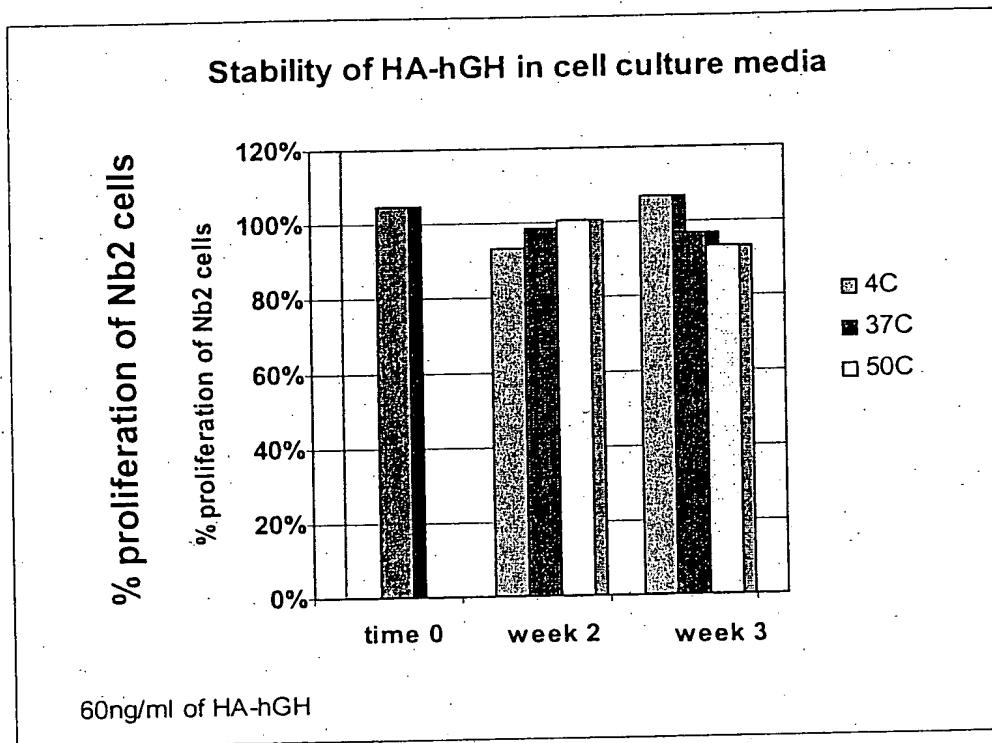


1/18



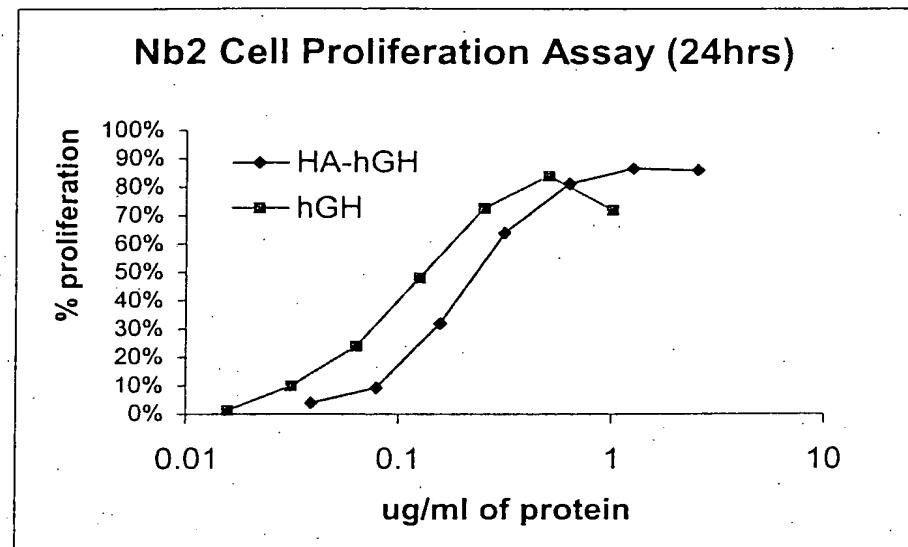
**Figure 1**

2/18

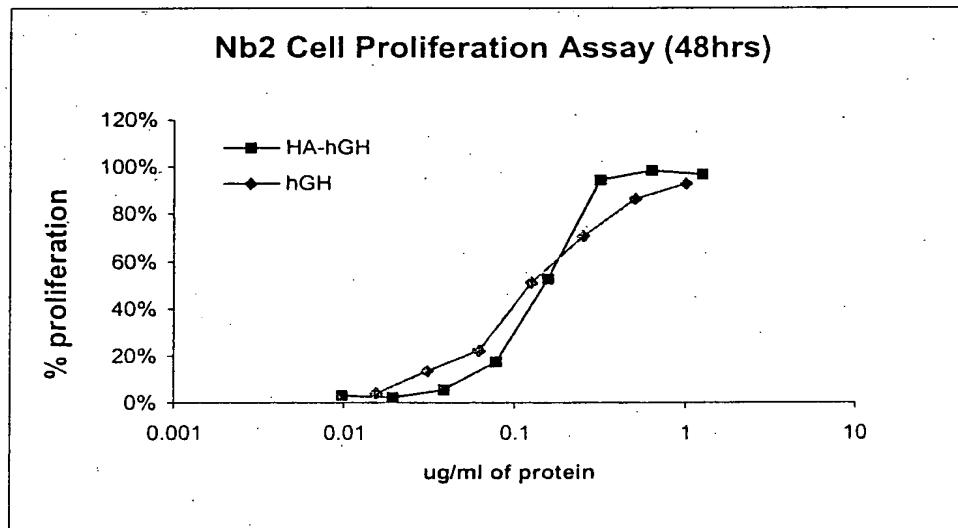


**Figure 2**

3/18



**Figure 3A**



**Figure 3B**

4/18

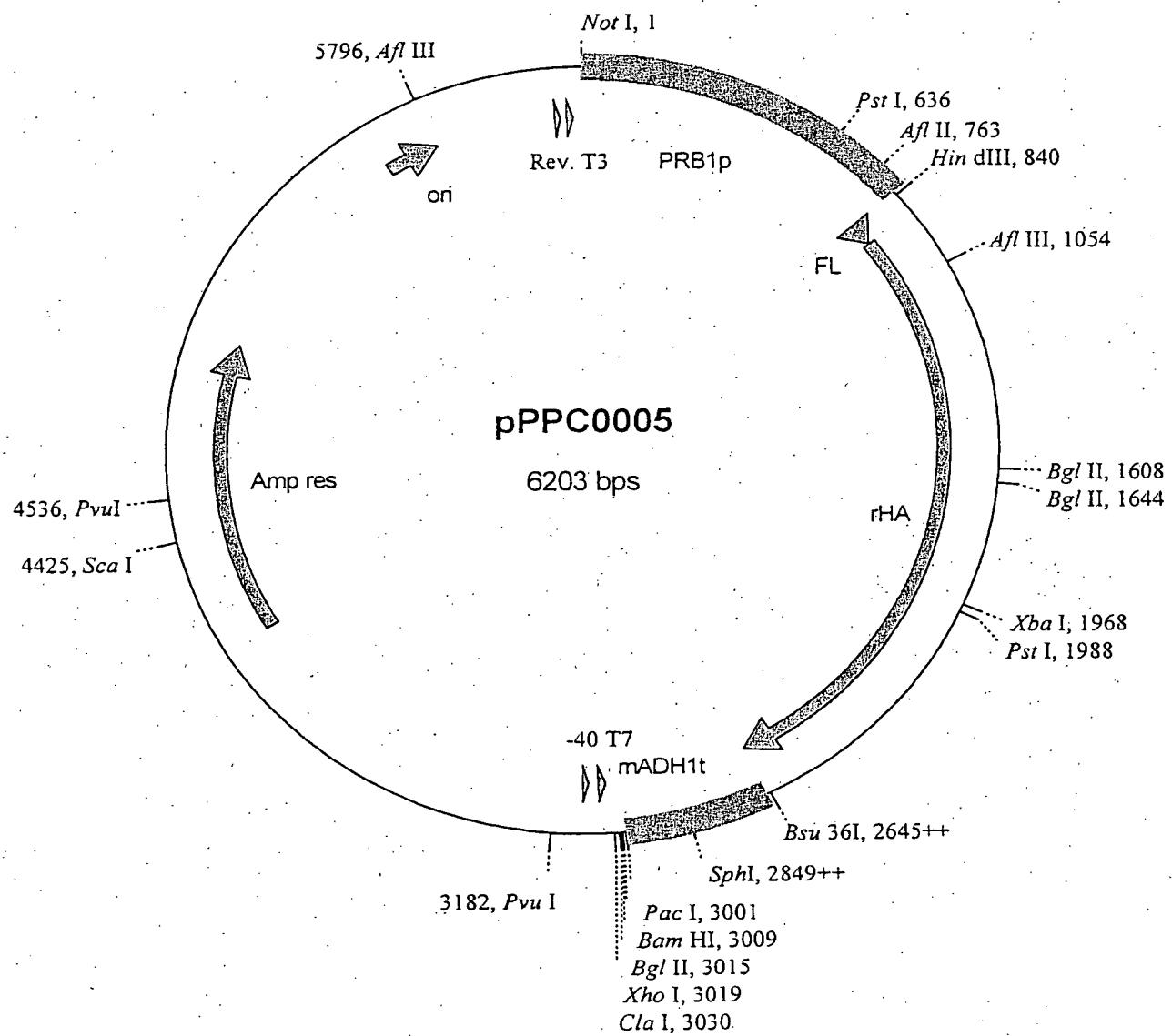


Figure 4

5/18

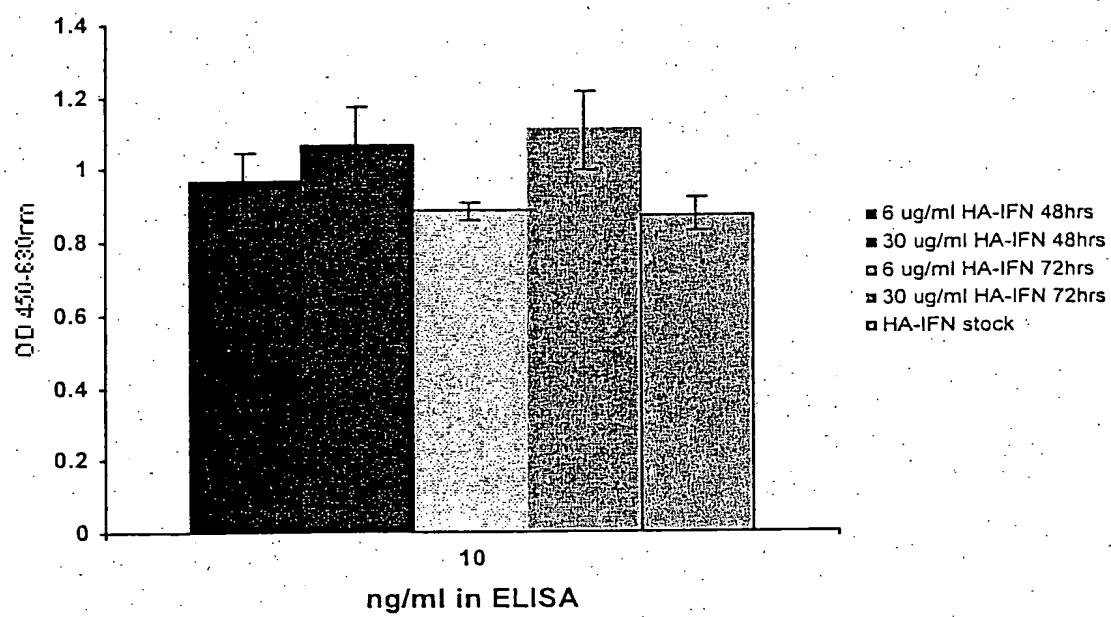


Figure 5

6/18

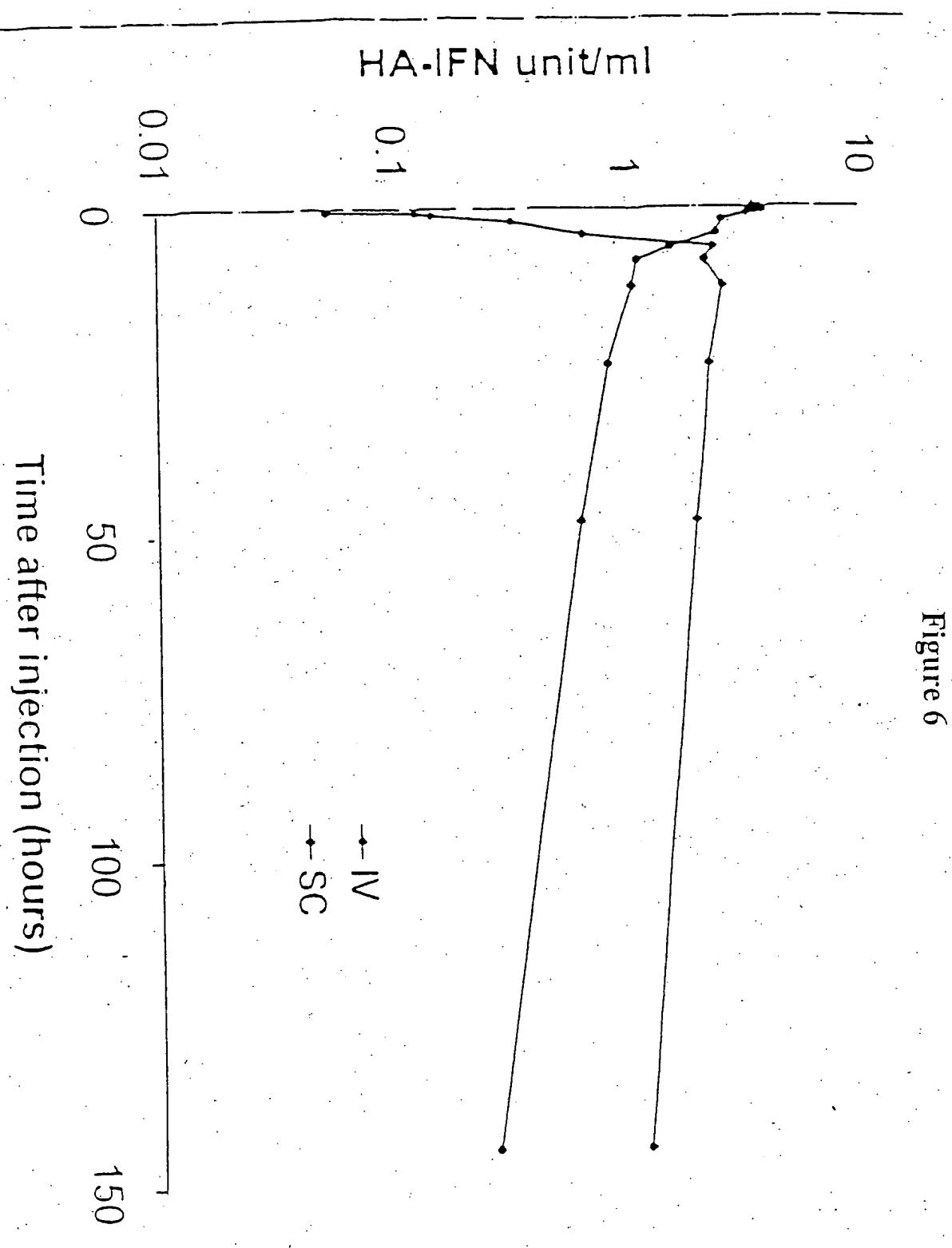


Figure 6

7/18

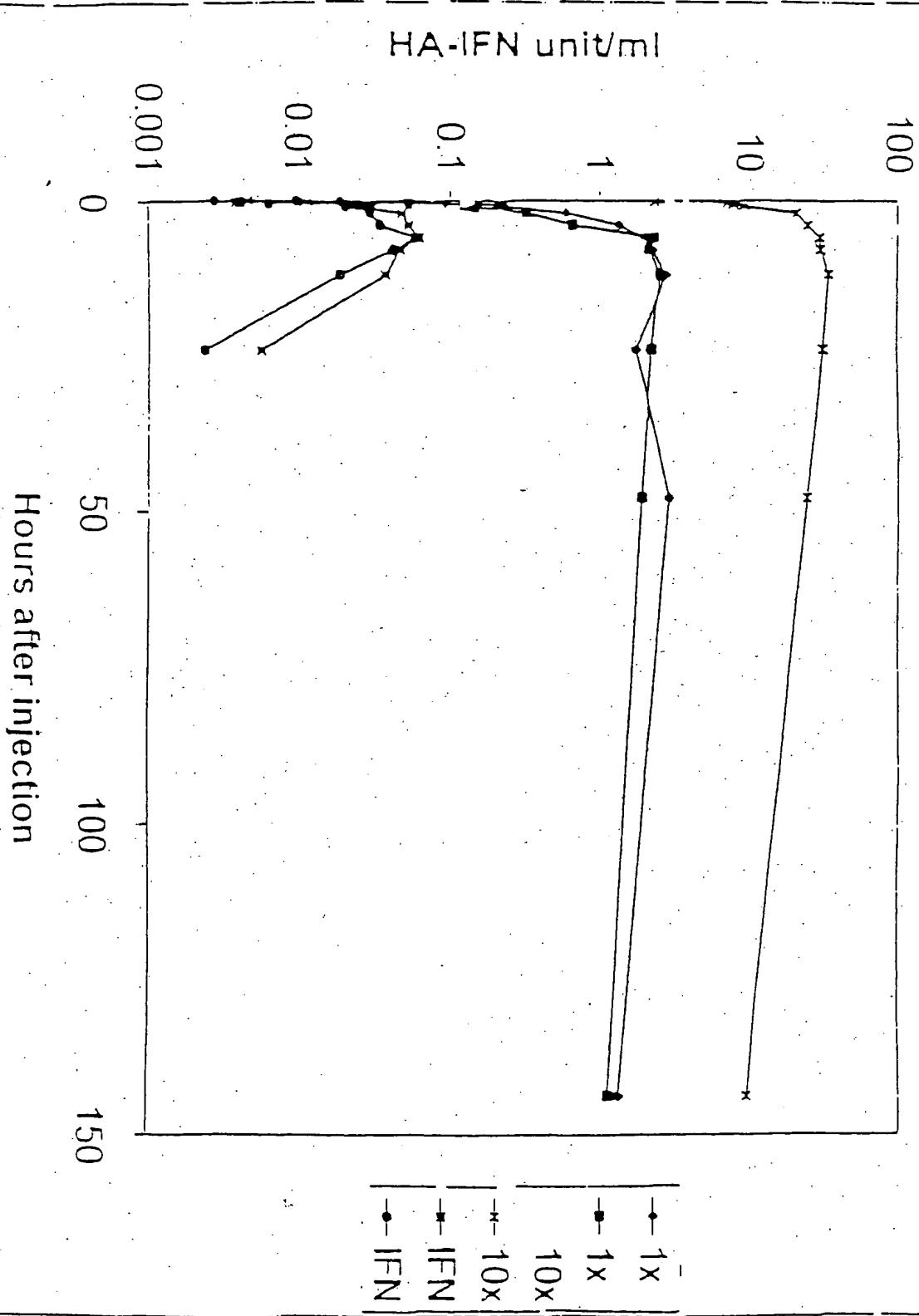
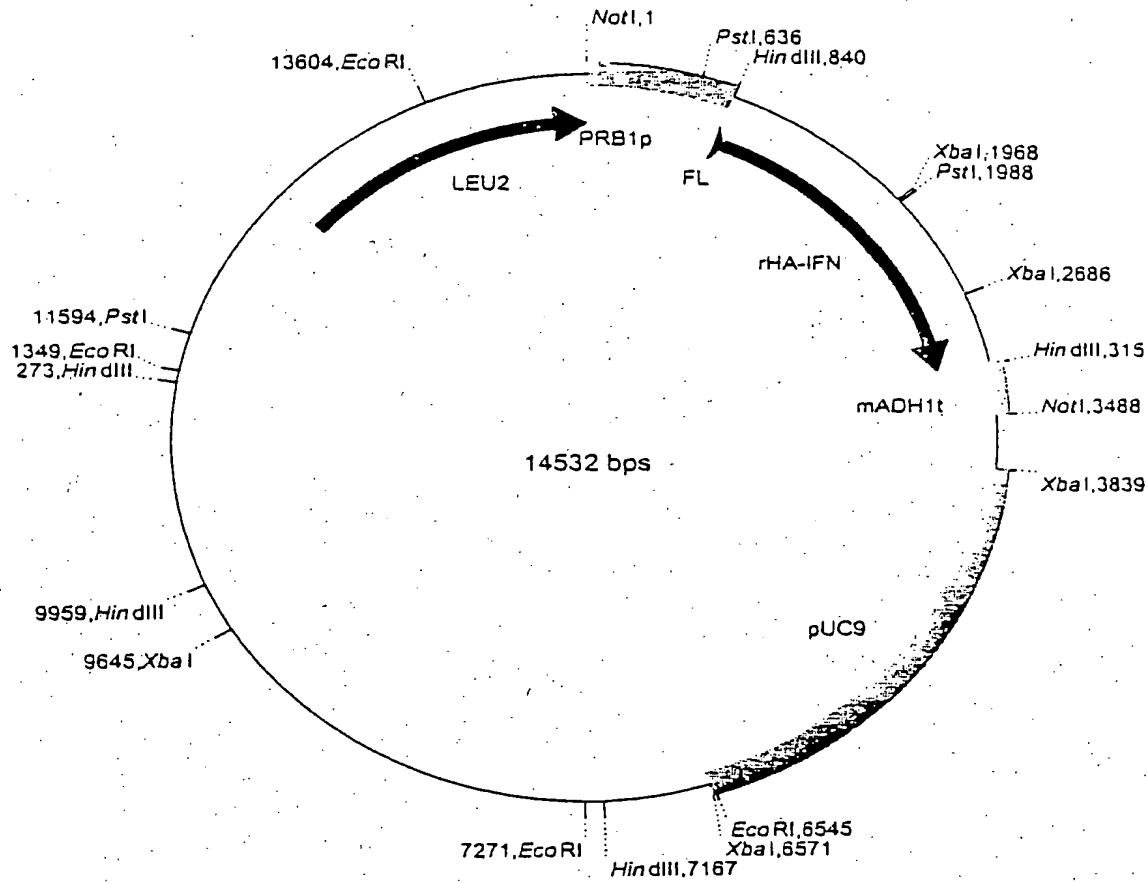


Figure 7



**Figure 8.** The HA-IFN $\alpha$  expression cassette in pSAC35. The expression cassette comprises

PRB1 promoter, from *S. cerevisiae*.

Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF $\alpha$ -1 leader.

HA-IFN $\alpha$  coding sequence with a double stop codon (TAATAA)

ADH1 terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the Hind III/BamHI fragment generally used.

**Figure 8**

9/18

## Localisation of 'Loops' based on the HA Crystal Structure which could be used for Mutation/Insertion

Loop		Loop	
I	Val54-Asn61	VII	Glu280-His288
II	Thr76-Asp89	VIII	Ala362-Glu368
III	Ala92-Glu100	IX	Lys439-Pro447
IV	Gln170-Ala176	X	Val462-Lys475
V	His247-Glu252	XI	Thr478-Pro486
VI	Glu266-Glu277	XII	Lys560-Thr566

**Figure 9**

10/18

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

IV

151 APELLFFFAKR YKAAFTECCQ AADKAACLLP KLDDELRDEGK ASSAKQRLKC  
HHHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH

IV

151 APELLFFFAKR YKAAFTECCX XXXXXXCLLP KLDDELRDEGK ASSAKQRLKC  
HHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

$(X)_n$

↓

IV

151 APELLFFFAKR YKAAFTECCQ AADKAACLLP KLDDELRDEGK ASSAKQRLKC  
HHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

11/18

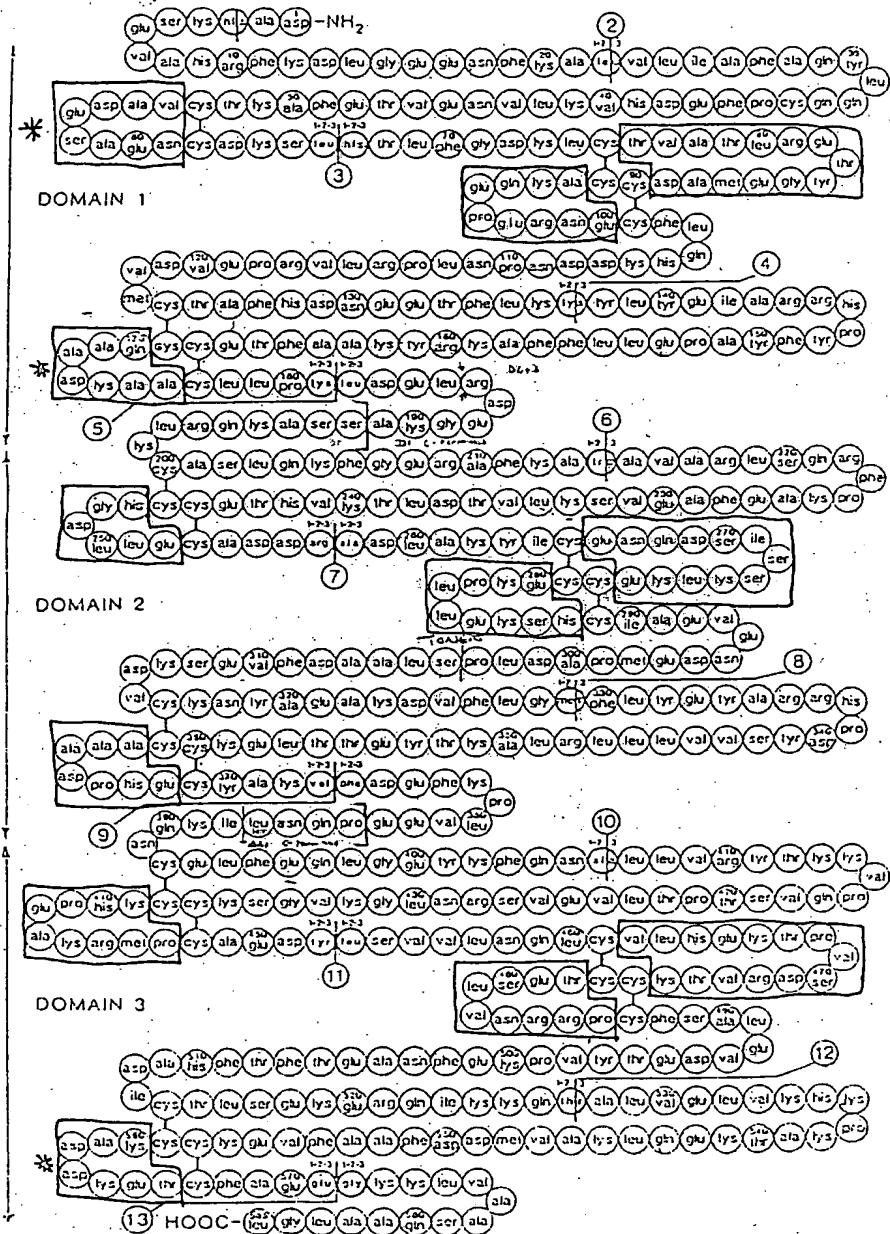


Figure 11

12/18

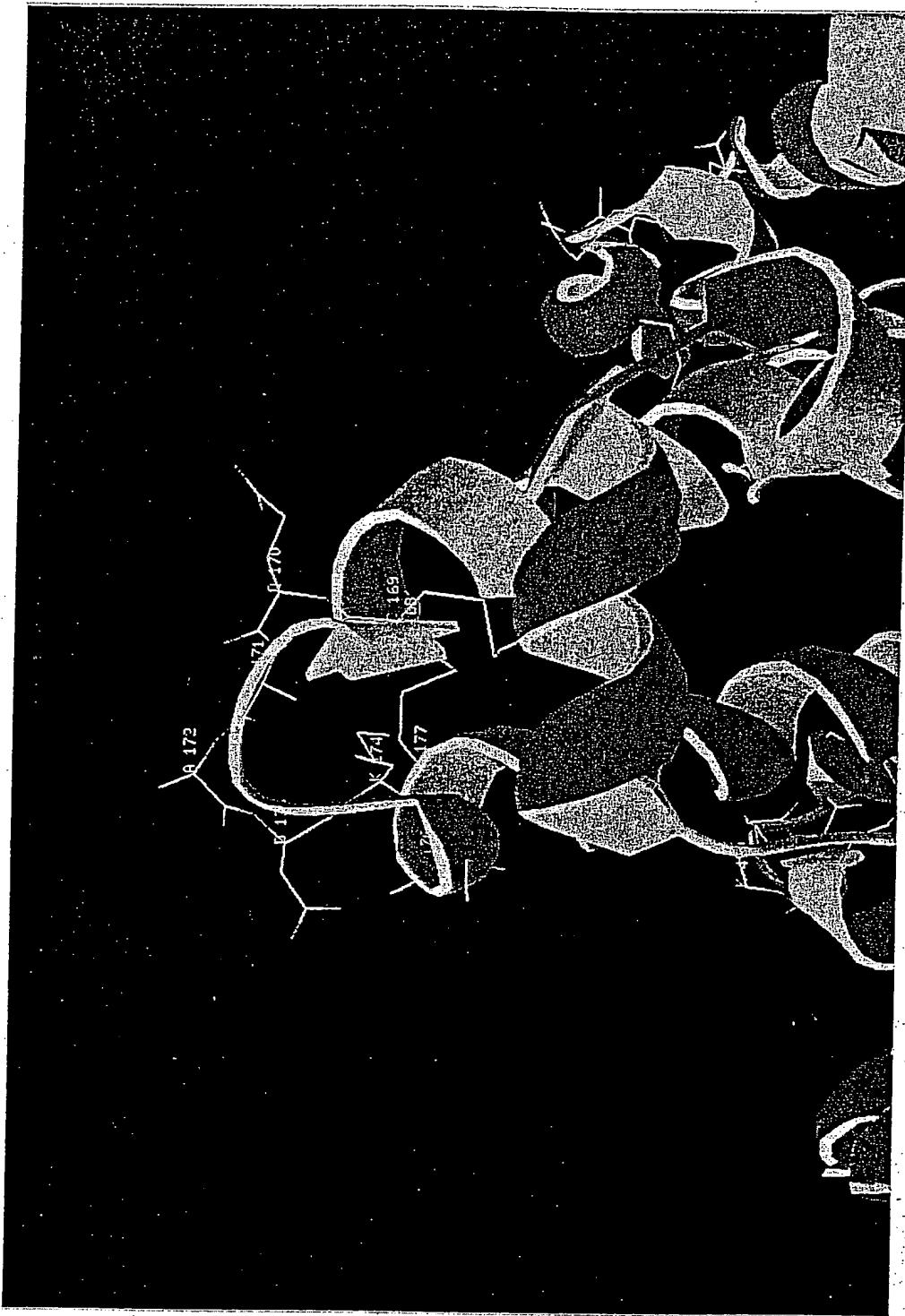


Figure 12: Loop IV Gln170-Ala176

13/18

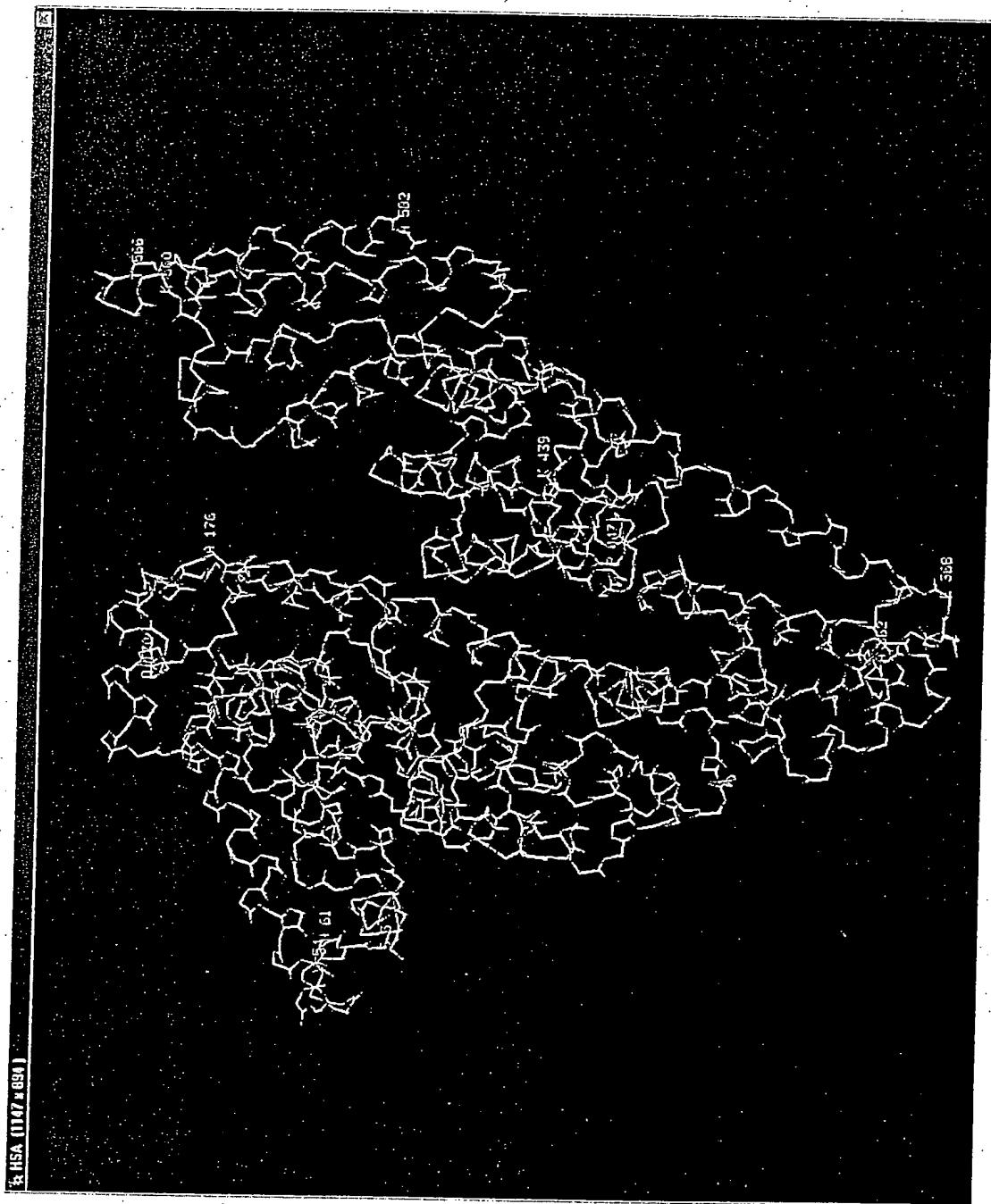


Figure 13: Tertiary Structure of HA

BEST AVAILABLE COPY

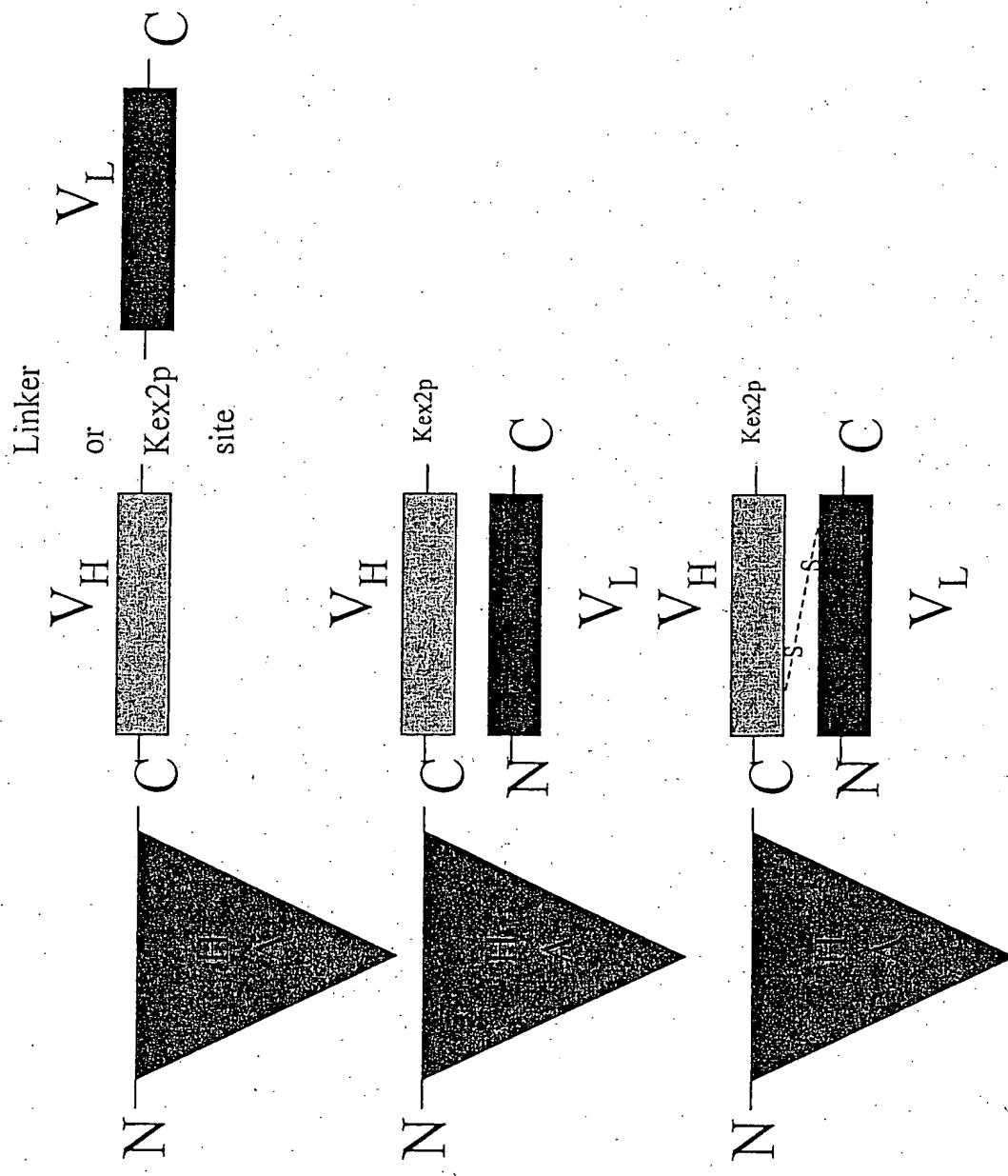


Figure 14: Schematic Diagram of Possible ScFv Fusions  
(Example is of a C-terminal fusion to HA)

15/18

1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TGT GGA GAA GAA AAT TTC AAA 60  
1 D A H K S E V A H R F K D L G E E N F K 20  
1 )  
  
61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120  
21 A L V L I A F A Q Y L Q Q C P F E D H V 40  
21 )  
  
121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180  
41 K L V N E V T E F A K T C V A D E S A E 60  
41 )  
  
181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240  
61 N C D K S L H T L F G D K L C T V A T L 80  
61 )  
  
241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300  
81 R E T Y G E M A D C C A K Q E P E R N E 100  
81 )  
  
301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360  
101 C F L Q H K D N P N L P R L V R P E V 120  
101 )  
  
361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420  
121 D V M C T A F H D N E E T F L K K Y L V 140  
121 )  
  
421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480  
141 E I A R R H P Y F V A P E L L F A K R 160  
141 )

Figure 15A

16/18

481 TAT AAA GCT TTT ACA GAA TGT TGC CAA GCT GAT AAA GCT GCC TGC CTG TTG CCA 540  
161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600  
181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660  
201 A S L Q K F P G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720  
221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780  
241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840  
261 A K Y I C E N Q D S I S S K L K E C C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA AAT GAT GAG ATG CCT GCT 900  
281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GCT GAT TTT GAT GAA ACT AAG GAT GTT TGC AAA AAC TAT GCT 960  
301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B

17/18

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020  
321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC GTG CTC CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080  
341 Y S V L L R L A K T Y E T L E K C 360

1081 TGT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT CTR 1140  
361 C A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200  
381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260  
401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC AAC CAG TTA 1320  
421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC AAC CAG TTA 1380  
441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440  
461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

18/18

1441 TGT GTG AAC AGC CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500  
481 L V N R R P C F S A L E V D E T Y V P K 500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560  
501 E F N A E T F T H A D I C T L S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620  
521 R Q I K K Q T A L V E L V K H K P K A T 540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680  
541 K E Q L K A V M D D F A A F V E K C C K 560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740  
561 A D D K E T C F A E E G K K L V A A S Q 580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782  
581 A L G L \* 585

Figure 15D